PCT WORLD PATENT ORGANIZATION
International Office
INTERNATIONAL PATENT APPLICATION PUBLISHED IN ACCORDANCE WITH
THE AGREEMENT ON INTERNATIONAL COOPERATION IN THE AREA OF PATENTS (PCT)

(51) International Patent Classification[7]: A61K 7/48 — A1

(11) International Publication No.: WO 00/15188

(43) International Publication Date: March 23 2000 (03/23/2000)

(21) International File No.: PCT/FR99/02178

(22) International Application Date: September 13 1999 (09/13/1999)

(30) Priority data:
98/11533 September 15, 1998 (09/15/1998) FR

(71) Applicant *(for all designated states except US)*: SEDERMA (FR/FR); 29 rue du Chemin Vert, Boite postale 33, F-78810 Le Perray en Yvelines (FR)

(72) Inventor; and
(75) Inventor/Applicant *(US only)*: LINTNER, Karl (FR/FR); 15 Avenue du Parc, F-78120 Rambouillet (FR)

(81) Designated states: AE, AL, AN, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, SI, GB,. GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU. LV, MD, MG, MK, MN, MW, ,\MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, European Patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL. PT, SE).

Published
With International Search Report.
Before the expiration of the period provided for the modification of claims, will be republished if modifications are received.

(54) Title: COSMETIC OR DERMOPHARMACEUTICAL USE OF PEPTIDES FOR HEALING, HYDRATING AND IMPROVING SKIN APPEARANCE DURING NATURAL OR INDUCED AGING (HELIODERMIA, POLLUTION)

(57) Abstract

The invention concerns the use of peptides of general sequence X-Thr-Thr-Lys-Y, wherein in particular X=lysine and Y=serine, in cosmetic or dermopharmaceutical compositions. It is moreover advantageous to use said peptides in mutual combination. In order to enhance their activity and their stability, the peptides are chemically modified to increase their lipophilicity, by grafting on the N-terminal amine of X, either a fatty acid chain, or by esterification or amidation of the C-terminal carboxyl group of Y. The peptides can be obtained by synthesis, biotechnology or controlled hydrolysis of plant proteins. The resulting compositions are advantageously used for stimulating healing, hydrating or all skin treatments. They are particularly active against formation or deterioration of wrinkles and against all the consequences of skin ageing, whether natural or induced (heliodermia, pollution), as well as for dry skin.

COSMETIC OR DERMOPHARMACEUTICAL USE OF PEPTIDES FOR HEALING, HYDRATING AND IMPROVING SKIN APPEARANCE DURING NATURAL OR INDUCED AGING (HELIODERMIA, POLLUTION)

Aging, particularly that of the skin, involves significant biochemical disturbances of intimate tissue which are materialized in macroscopic modifications, usually judged to be unsightly, and which have persistently preoccupied both women and men.

The pursuit of a suntan using natural or solar UV or by the artificial UV of *beauty parlors*, is responsible for a process of skin aging well-known to dermatologists by the name *heliodermia* (Dr. C. Musy-Preault, Diseases of the Skin, 1994, Albin Michel ed., Paris).

Other components of our current lifestyle, such as the physical and chemical assaults of pollution and the consumption of alcohol and tobacco, promote and exacerbate the aging processes.

Moreover, in the course of private or professional life, the skin, the first defense of the organism with regard to the outside world, is threatened in its integrity by numerous localized attacks such as cuts, burns, and inflammatory reactions. To correct their damage, the organism has developed a number of complex and mutually overlapping reactions: healing.

The cosmetic industry is continually searching for new ingredients capable of countering the effects of aging in general and/or promoting cutaneous healing.

For this, one of the possible approaches consists of promoting tissue restructuring by the neosynthesis of the various constituent elements of the skin. Just like the cement which assures the cohesion of the bricks in a wall and imparts its solidity to it, the different types of collagen and other mucopolysaccharides are the constituent elements of cutaneous tissue.

Promotion of the synthesis and incorporation of these substances is certainly essential, but is not in itself sufficient. The *terrain must be prepared* by giving it a good foundation on which the mechanisms of healing will be able to effectuate lasting repairs. In the situations described above, this foundation is the extracellular matrix, which takes the name basal layer when it is situated at the interface of the epithelium and the connective tissue. The improvement or reconstruction of the extracellular matrix is essential because it is now known that not only does this structure act "*as a frameork, stabilizing the physical structure of the tissues*," but it also "*plays a part ... in the regulation of the behavior of the cells that are in contact with it - influencing their development, migration, proliferation, form and functions*" (Molecular Biology of the Cell, 3rd ed., Medecine-Sciences, Flammarion, Paris, page 972).

We were thus especially interested in two of the main constituents of this extracellular matrix: the collagens and the glycosaminoglycans (also known by the name GAGs).

Within the framework of this patent, the effects of aging on the collagens and the glycosaminoglycans can be summarized by:

> The <u>*decrease in the synthesis*</u> of these substances by the fibroblasts, a decrease due to the conjunction of two causes: on one hand, the rate of renewal of these productive cells decreases with age, and, on the other, the quantity of substances secreted by these cells likewise diminishes.

When it is known that collagen represents about 80% of the cutaneous proteins, it is easy to understand that the slightest decrease in its tissue concentration can have important consequences on the mechanical and physiological properties of the skin.

The glycosaminoglycans are capable of fixing large amounts of water. The drop in their tissue concentration is thus accompanied by cutaneous dehydration.

The *appearance of structural modifications* of the neosynthesized substances which lead to the cross-linking of the fibers and thus to their becoming rigid.

For collagen, the variations in the α chains modify the distribution of its various forms. For example, the proportion of Type III collagen increases in the epidermis when Type IV collagen accumulates in the basal membrane. The appearance of reactions, enzymatic or not (of the Maillard reaction type), is also observed that create linkages, called crossed linkages, either between two collagen fibers or between the collagen itself and molecules of glucose, thus making the networks of collagen fiber rigid.

Aging is expressed in the glycosaminoglycans by the imperfect synthesis of their polysaccharide chains and by a decrease in their sulfation. Even more than with collagen, the free radical forms of oxygen degrade the GAGs irreversibly.

The skin thus loses some of its *substance* due to the decrease in the quantity of its constituents and hardens due to the loss of elasticity of the collagen fibers and due to its dehydration.

All of this contributes to giving old skin its characteristic appearance: dryness, lack of smoothness, thinning, fragility, and wrinkles of varying number and depth.

As for healing, this at least in part involves similar needs since it must reconstruct and thus build up the tissue mass; locally, this involves the increased synthesis of various cutaneous constituents.

Thus, any product capable of inducing one or more processes that increase locally the synthesis of collagens and glycosaminoglycans will permit the effect sought by all those wishing to reduce the cutaneous stigmata of aging as well as by those wishing to improve healing both with regard to time and with regard to the esthetic appearance and quality of the result.

The invention that is the subject of this patent application is based on the fact that we have developed a product that responds to the above criteria and that we have demonstrated its efficacy in vitro and in vivo by sophisticated scientific tests.

It is known that the synthesis of collagen can be stimulated (in vitro), in cell cultures, by the C-terminal fragment of collagen I which is constituted by the peptide Lys-Thr-Thr-Lys-Ser (Katayama, K., et al., Journal of Biological Chemistry, 259, 9941-9944 (1993)).

Moreover, it is possible to increase the synthesis of the cutaneous glycosaminoglycans by means of plant extracts (for example, in the rat: Chithra, P., et al., Journal of Ethnopharmacology, 59, 179-186 (1998)).

Our patent application is based on the discovery that, administered alone or in combination by the topical route in vivo, and thus in an approach relevant to cosmetics, the peptides of general formula R_1-X-Thr-Thr-Lys-$(AA)_n$-Y and their salts, with:

- X representing a basic amino acid of D or L form (lysine, arginine, histidine, ornithine, citrulline, sarcosine, statine),

- $(AA)_n$ representing a chain of n amino acids, natural or not, with n varying between 0 and 5,

- R_1 being H or a fatty acid chain with 2 to 22 carbons, hydroxylated or not, saturated or not, linear or branched, containing sulfur or not, cyclic or not, or a biotinyl group, or a protective group of the urethane type used in peptide synthesis such as the benzyloxycarbonyl (Z), terbutyloxycarbonyl (tBoc), fluorenylmethyloxycarbonyl (Fmoc), allyloxycarbonyl (Alloc) groups,

- Y = OR_2 or $N_2R_2R_3$ with R_2 and/or R_3 being a hydrogen atom H or an aliphatic or aromatic chain of 1 to 22 carbons, hydroxylated or not, saturated or not, linear or branched, containing sulfur or not, cyclic or not,

with the exception of peptides with R_1 = H and X = Lys and Y = OH and with n = 0 or $(AA)_n$ = Ser when n = 1,

are capable of increasing very considerably the concomitant synthesis of collagen and that of the glycosaminoglycans and that this fact permits a synergic effect to be obtained since the result observed is better than that which would have been expected from the addition of each of these effects.

In fact, the newly formed collagen fibers are immediately interlinked into the network of the glycosaminoglycans of the newly synthesized basal layer, thus accelerating the process of cutaneous regeneration as well as the average level of tissue hydration.

The peptides used advantageously from this point of view can be characterized by the fact that n = 1, R_1 is a fatty acid chain with 2 to 22 carbons, and Y is OH or NH_2, and more precisely with X = lysine, $(AA)_n$ = serine, R_1 = a palmitoyl group and Y = OH.

The peptides that are the subjects of this patent application can be obtained either by standard chemical synthesis (in heterogeneous or homogeneous phase), or by enzymatic synthesis (Kullman et al., J. Bio. Chem. 255, 8234 (1980)) from the constituent amino acids or their derivatives.

The small size of these peptides makes it possible to carry out their industrial synthesis at an advantageous cost. Their demonstrated high activity permits their commercial use in a large number of financially acceptable cosmetic or dermopharmaceutical products.

The peptides can also be obtained by fermentation of a strain of bacteria, whether or not modified by genetic engineering, to produce the desired sequences or their different fragments.

Finally, the peptides can be obtained by extraction of proteins of animal or plant origin, preferably of plant origin, capable of containing these sequences within their structure, followed by controlled enzymatic or non-enzymatic hydrolysis, which liberates the peptide fragments in question (of the sequence X-Thr-Thr-Lys-(AA), preferably Lys-Thr-Thr-Lys-Ser), of average size between 300 and 2000 daltons, with the stipulation that the liberated fragments correspond to the above peptide sequence in the plants that are capable of containing these sequences within their structure. Controlled hydrolysis permits the liberation of these peptide fragments.

To implement the invention it is possible, but not necessary, either to extract the proteins involved first and then hydrolyze them, or to effectuate the hydrolysis first on a crude extract and then to purify the peptide fragments. The hydrolysate can also be used without extracting from it the peptide fragments in question.

nevertheless making sure of the halting of the enzymatic hydrolysis reaction in time and to assay the presence of the peptides in question by appropriate analytical means (tracing by radioactivity, immunofluorescence or immunoprecipitation with specific antibodies, etc.).

Other simpler or more complex processes leading to cheaper or purer products can easily be envisaged by one skilled in the art who is knowledgeable about the technique of extraction and purification of proteins and peptides.

As an example to illustrate the invention, some cosmetic formulas that represent but do not limit the invention are cited:

Example No. 1: Gel

Carbopol 1342®	0.3
Propylene glycol	2.0
Glycerine	1.0
White petrolatum	1.5
Cyclomethicone	6.0
Cetyl alcohol	0.5
Lubrajel® MS	10
Triethanolamine	0.3
N-Palmitoyl-sarcosine-Lys-Thr-Thr-Lys-Ser	0.0005
Water, preservatives, perfume	qsp 100 g.

Example No. 2: Cream

Volpo S20	2.4
Volpo S2	2.6
Prostearyl 15	8.0
Beeswax	0.5
Abil® ZP 2434	3.0
Propylene glycol	3.0
Carbopol® 941	0.25
Triethanolamine	0.25
N-Palmitoyl-Lys-Thr-Thr-Lys-Ser	0.005
Water, preservatives, perfume qsp	100 g

The activities described at the beginning of this application are illustrated by the following examples.

Example No. 3 Increase in the synthesis of collagen: *in vitro*

The method chosen is a variant of that described by Augustin, C., et al. (Skin Pharmacol. 10, 63-70 (1997)) in that we have used explants of human skin instead of human pulmonary fibroblasts in order to make our results directly usable in cosmetology.

These explants, from mammary or abdominal plastic surgery, are incubated for 72 hours in the presence of ^{3}H-proline with the peptide N-palmitoyl-sarcosine-Thr-Thr-Lys-Ser, in three final concentrations in the culture medium (2×10^{-4}%, 4×10^{-4}% and 8×10^{-4}%; that is, 2, 4 and 8 ppm). The explants are then washed, the dermis and epidermis of each explant are separated, homogenized and subjected to lysis. The measurement of the incorporation of ^{3}H-proline is then carried out in each lysate. The tests are performed in triplicate.

In parallel, *negative* controls are effectuated under the same conditions but in the absence of the peptide. *Positive* controls effectuated by replacing the peptide tested with Vitamin C.

In the presence of 2, 4 or 8 ppm peptide, the incorporation of ^{3}H-proline, which expresses the synthesis of collagen, is raised by respectively 30.2 (± 2)%, 54.7 (± 5)% and 90.9 (± 5)% relative to that which is observed in the control experiments (without the peptide).

Under the same conditions, the reference product, ascorbic acid at the concentration of 0.5 mM, increases the synthesis of collagen by 61.4 (± 5)%.

Example No. 4: Increase in the synthesis of glycosaminoglycans: *in vitro*
The same protocol as that in Example No. 3 is used, except that on one hand the incubation is carried out in the presence of ^{3}H-glucosamine in place of the ^{3}H-proline and on the other that Vitamin A acid is used as reference product in place of Vitamin C.

In the presence of 2, 4 or 8 ppm of the peptide Lys-Thr-Thr-Lys-Ser-Ala, the incorporation of ^{3}H-glucosamine, which expresses the synthesis of GAGs, is increased respectively by 24.5 (± 3)%, 48.8 (± 3)% and 67.9 (± 5)% relative to that which is observed in the control experiments (without the peptide).

Under the same conditions, the reference product, Vitamin A acid at the concentration of 100 nM, increases the synthesis of GAGs by 45.3 (± 2)%.

The results obtained in these two examples clearly demonstrate a concentration-dependant effect of the peptide on the synthesis of the two constituents of the extracellular matrix.

Example No. 5: Skin firming activity: *in vitro*
Over 24 hours, human fibroblasts from the same cell culture are put in the presence of standard culture medium, supplemented with different concentrations of peptide (2, 4 and 8 ppm). The controls were unsupplemented.

The stimulation of the synthesis of proteins is evaluated by colorimetry (Biuret reaction).

To standardize the results, the quantity of proteins measured is expressed for 1000 cells present in the test.

Relative to the control experiments, in the presence of either 2, 4 and 8 ppm of the peptide N-palmitoyl-Lys-Thr-Thr-Lys-Ser, the rise in the concentration of proteins is respectively 14.7 (± 1.0)%, 21.0 (± 2.4)% and 44.8 (± 1.0)%.

Thus, this *in vitro* test demonstrates the concentration-dependant stimulation potential of the peptide at the cutaneous level, an effect directly linked to a firming and thickening of skins that are too thin.

Example No. 6: Anti-wrinkle activity: *in vivo*
This example reports the anti-wrinkle effect obtained in vivo on a panel constituted of 15 adult female volunteers aged 35 to 63 years. The anti-wrinkle effect of the cream from Example No. 2, containing N-palmitoyl-Lys-Thr-Thr-Lys-Ser at the concentration of 0.005% (50 ppm), is compared with that of a placebo cream (same cream but without the active substance). The creams are applied to precisely identified sites located at the corner of the right or left eye, according to a randomized distribution, twice a day for 28 days. The parameter used is the cutaneous relief at the level of the contour of the eye (the wrinkles known as crow's feet). The quantifications of the different relief variables are carried out by video data processing analysis of silicone imprints taken at the surface of the skin according to protocols described by Corcuff et al. (Int. J. Cosm. Sci. 7, 117-126 (1985)) and Corcuff et al. (in Handbook of non-invasive methods and the skin, Serup & Jemec, Eds., CRC Press, 1985, pp. 89-96).

The table below indicates the difference, in percentages, in the average values obtained between T + 28 days and T0 for the average depths of the principal wrinkle (column A) or for all the wrinkles (column B); for the density of the main wrinkles (column C) as well as for the measurement of the roughness (column D).

	A	B	C	D
Placebo				

The cream containing N-palmitoyl-Lys-Thr-Thr-Lys-Ser previously described clearly demonstrates a powerful anti-wrinkle effect since a significant difference is observed between the start and finish of the in vivo study, on the total of the four parameters commonly used in this indication.

It is to be noted that under the same experimental conditions, the placebo cream shows no effect if the N-palmitoyl-Lys-Thr-Thr-Lys-Ser was not incorporated, which demonstrates well that the beneficial effect observed can be attributed only to the peptide that is the subject of this patent.

It is particularly advantageous to use these peptides in combination.

The peptides of this patent can be obtained by chemical synthesis, by the enzymatic route, by fermentation, by extraction of proteins of plant origin, by standard peptide synthesis in homogeneous or heterogeneous phase or by enzymatic synthesis starting from the constituent amino acids.

The peptides of this patent can be obtained by extraction of proteins from plants, followed by hydrolysis, enzymatic or non-enzymatic, so as to generate peptide fragments of average size between 300 and 2000 daltons; part of the liberated fragments must contain the sequence X-Thr-Thr-Lys-$(AA)_n$, preferably the sequence Lys-Thr-Thr-Lys-Ser.

The peptides in this patent, alone or in combination, can be used at concentrations varying between 0.1 and 1000 ppm (w/w), preferably between 1 and 100 ppm (w/w) in the finished cosmetic or dermopharmaceutical product.

The peptides of this patent, alone or in combination, can be used in the form of a solution, dispersion, or emulsion, or encapsulated in vectors such as macro-, micro- or nanocapsules, liposomes or chylomicrons, or included in macro-, micro- or nanoparticles, or in microsponges, or adsorbed on to powdered organic polymers, talcs, bentonites and other mineral supports.

The peptides of this patent, alone or in combination, can be used in any galenical form: O/W and W/O emulsions, milks, lotions, gelling and viscosing polymers, surfactants and emulsifiers, pomades, hair lotions, shampoos, soaps, powders, sticks and crayons, sprays, and body oils.

The peptides of this patent, alone or in combination, can be used with any other commonly used ingredient: extraction and/or synthesis lipids, gelling and viscosing polymers, surfactants and emulsifiers, water- or liposoluble active principles, plant extracts, tissue extracts, marine extracts, sun screens, and antioxidants.

The peptides of this patent, alone or in combination, are used in cosmetic applications to promote healing, hydration, and for all care of the skin, particularly against the formation and exacerbation of wrinkles and against all the consequences of natural or accelerated aging of the skin (heliodermia, pollution).

The peptides of this patent, alone or in combination, as well as the cosmetic and dermopharmaceutical compositions containing them, are used for the preparation of a medication to promote healing, hydration and for all skin care, particularly against the formation and exacerbation of wrinkles and against all the consequences of the natural or accelerated aging of the skin (heliodermia, pollution).

Claims

1 Peptides of the general formula R_1-X-Thr-Thr-Lys-$(AA)_n$-Y and their salts, with:

- X representing a basic amino acid of D or L form (lysine, arginine, histidine, ornithine, citrulline, sarcosine, statine),
- $(AA)_n$ representing a chain of n amino acids, natural or not, with n varying between 0 and 5,
- R_1 being H or a fatty acid chain of 2 to 22 carbons, hydroxylated or not, saturated or not, linear or branched, containing sulfur or not, cyclic or not, or a biotinyl group, or a protective group of the urethane type used in peptide synthesis such as the benzyloxycarbonyl (Z), terbutyloxycarbonyl (tBoc), fluorenylmethyloxycarbonyl (Fmoc), and allyloxycarbonyl (Alloc) groups,
- Y = OR_2 or $N_2R_2R_3$ with R_2 and/or R_3 being a hydrogen atom H or an aliphatic or aromatic chain of 1 to 22 carbons, hydroxylated or not, saturated or not, linear or branched, containing sulfur or not, cyclic or not,

with the exception of the peptides with R_1 = H and X = Lys and Y = OH and with n = 0 or $(AA)_n$ = Ser when n = 1.

2 Peptides in accordance with claim 1, characterized by the fact that n = 1, R_1 is a fatty acid chain with 2 to 22 carbons and Y is OH or NH_2.

3. Peptide in accordance with claim 2,characterized by the fact that X is lysine, $(AA)_n$ is serine, R_1 is the palmitoyl group and Y is the OH group.

4. Use of peptide(s) of the general formula R_1-X-Thr-Thr-Lys-$(AA)_n$-Y and their salts, with: X representing a basic amino acid of D or L form (lysine, arginine, histidine, ornithine, citrulline, sarcosine, statine), $(AA)_n$ representing a chain of n amino acids, natural or not, with n varying between 0 and 5, and R_1 = H or a fatty acid chain of 2 to 22 carbons, hydroxylated or not, saturated or not, linear or branched, containing sulfur or not, cyclic or not, or a biotinyl group, or a protective group of the urethane type used in peptide synthesis such as the benzyloxycarbonyl (Z), terbutyloxycarbonyl (tBoc), fluorenylmethyloxycarbonyl (Fmoc), and allyloxycarbonyl (Alloc) groups, Y = OR_2 or $N_2R_2R_3$ with R_2 and/or R_3 being a hydrogen atom H or an aliphatic or aromatic chain of 1 to 22 carbons, hydroxylated or not, saturated or not, linear or branched, containing sulfur or not, cyclic or not, alone or in combination, in cosmetic or dermopharmaceutical compositions.

5. Cosmetic or dermopharmaceutical compositions in accordance with claim 4, characterized by the fact that the peptide(s) is(are) obtained by chemical synthesis, by the enzymatic route, by fermentation or by extraction of proteins of plant origin.

6. Cosmetic or dermopharmaceutical compositions in accordance with claims 4 and 5, characterized by the fact that the peptide(s) is(are) obtained by standard peptide synthesis in homogeneous or heterogeneous phase or by enzymatic synthesis starting from the constituent amino acids or their derivatives.

7 Cosmetic or dermopharmaceutical compositions in accordance with claims 4 to 6, characterized by the fact that the peptide(s) is(are) obtained by extraction of proteins from plants, followed by enzymatic or non-enzymatic hydrolysis, in such a way as to generate peptide fragments of average size between 300 and 2000 daltons, part of the fragments liberated having to contain the sequence X-Thr-Thr-Lys-$(AA)_n$, preferably the sequence Lys-Thr-Thr-Lys-Ser.

8. Cosmetic or dermopharmaceutical compositions in accordance with claims 4 to 7, characterized by the fact that the peptide(s) is(are) used at concentrations varying between 0.1 and 1000 ppm (w/w), preferably between 1 and 100 ppm (w/w) in the finished product.

9. Cosmetic or dermopharmaceutical compositions in accordance with claims 4 to 8, characterized by the fact that the peptide(s) is(are) used in the form of a solution, dispersion, or emulsion, or encapsulated in vectors like macro-, micro- or nanocapsules, liposomes or chylomicrons, or included in macro-, micro- or nanoparticles, or in microsponges, or adsorbed on powdered organic polymers, talcs, bentonites and other mineral supports.

10. Cosmetic or dermopharmaceutical compositions in accordance with claims 4 to 9, characterized by the fact that the peptide(s) is(are) used in any galenical form: O/W and W/O emulsions, milks, lotions, gelling and viscosing polymers, surfactants and emulsifiers, pomades, hair lotions, shampoos, soaps, powders, sticks and crayons, sprays, and body oils.

11 Cosmetic or dermopharmaceutical compositions in accordance with claims 4 to 10, characterized by the fact that the peptide(s) is(are) used with any other commonly used ingredient: extraction and/or synthesis lipids, gelifying and viscosing polymers, surfactants and emulsifiers, water- or liposoluble active principles, plant extracts, tissue extracts, marine extracts, sun screens, and antioxidants.

12. Cosmetic or dermopharmaceutical compositions in accordance with claim 4 to 11, used in cosmetic applications to promote healing, hydration, and for all skin care, particularly against the formation and exacerbation of wrinkles as well as against all the consequences of natural or accelerated aging of the skin (heliodermia, pollution).

13. Use of peptides in accordance with the general formula R_1-X-Thr-Thr-Lys-$(AA)_n$-Y and their salts, with: X representing a basic amino acid of D or L form (lysine, arginine, histidine, ornithine, citrulline, sarcosine, statine), $(AA)_n$ representing a chain of n amino acids, natural or not, with n varying between 0 and 5, and R_1 = H or a fatty acid chain of 2 to 22 carbons, hydroxylated or not, saturated or not, linear or branched, containing sulfur or not, cyclic or not, or a biotinyl group, or a protective group of the urethane type used in peptide synthesis such as the benzyloxycarbonyl (Z), terbutyloxycarbonyl (tBoc), fluorenylmethyloxycarbonyl (Fmoc), and allyloxycarbonyl (Alloc) groups, and Y = OR_2 or $N_2R_2R_3$ with R_2 and/or R_3 being a hydrogen atom H or an aliphatic or aromatic chain of 1 to 22 carbons, hydroxylated or not, saturated or not, linear or branched, containing sulfur or not, cyclic or not, alone or in combination; or of cosmetic or dermopharmaceutical compositions in accordance with claim 4 to 12, for the preparation of a medication to promote healing, hydration, and for all skin care, particularly against the formation and exacerbation of wrinkles as well as against all the consequences of natural or accelerated aging of the skin (heliodermia, pollution).

INTERNATIONAL SEARCH REPORT

Interr nal Application No
PCT/FR 99/02178

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K7/48

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	K. KATAYAMA E.A.: "A Pentapeptide from Type I Procollagen Promotes Extracellular Matrix Production" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 268, no. 14, 1993, pages 9941-9944, XP002106610 cited in the application page 9941	1,4

☐ Further documents are listed in the continuation of box C.

☐ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 January 2000	31/01/2000

Name and mailing address of the ISA
European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer
Peeters

Form PCT/ISA/210 (second sheet) (July 1992)